CLAIMS

The subject matter claimed is:

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- 1. A composition of matter having a formula represented by ${\rm H_3C\text{-}NH\text{-}}\left({\rm CH_2\text{-}CH_2\text{-}NH}\right)_x\text{-}{\rm CH_2\text{-}X\text{-}Y}$
- wherein x is an integer of about 8 to about 1,200, X is a linker, and Y is a residue of a sterol comprising a 3-ol group.
 - 2. The composition of matter of claim 1 wherein x is about 581.
- 3. The composition of matter of claim 1 wherein X is -0-CO-.
 - 4. The composition of matter of claim 1 wherein Y is a cholesterol residue.
 - 5. The composition of matter of claim 1 wherein Y is a member selected from the group consisting of residues of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmasterol, allocholesterol, 24-hydroxycholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -sitosterol, β -sitosterol, γ -sitosterol,

lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.

- 6. A composition of matter having a formula represented by $H_3C-NH-(CH_2-CH_2-NH)_x-CH_2-O-CO-Y$
- wherein x is an integer of about 8 to about 1,200, and Y is a cholesterol residue.
 - 7. The composition of matter of claim 6 wherein x is about 581.
- wherein x is an integer of about 0 to about 1,200, y is an integer of about 0 to about 1,200, with the proviso that x + y is about 8 to about 1,200, X is a linker, and Y is a residue of a sterol comprising a 3-ol group.
- 9. The composition of matter of claim 8 wherein x + y is about 581.
 - 10. The composition of matter of claim 8 wherein X is -CO-.

- 11. The composition of matter of claim 8 wherein Y is a cholesterol residue.
- 12. The composition of matter of claim 8 wherein Y is a member selected from the group consisting of residues of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmasterol, stigmastanol, (3β) -7-dehydrocholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -sitosterol, β -sitosterol, γ -sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.

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wherein x is an integer of about 0 to about 1,200, y is an integer of about 0 to about 1,200, with the proviso that x + y is about 8 to about 1,200, and Y is a cholesterol residue.

14. The composition of matter of claim 13 wherein x + y is about 581.

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wherein x is an integer of about 0 to about 1,200, y is an integer of about 0 to about 1,200, with the proviso that x + y is about 8 to about 1,200, X_1 and X_2 are linkers, and Y_1 and Y_2 are residues of a sterol comprising a 3-ol group.

- 16. The composition of matter of claim 15 wherein x + y is about 581.
- 17. The composition of matter of claim 15 wherein X_1 is -O-CO- and X_2 is -CO-.
- 18. The composition of matter of claim 15 wherein Y_1 and Y_2 are cholesterol residues.
 - 19. The composition of matter of claim 15 wherein Y_1 and Y_2 are members independently selected from the group consisting of residues of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -
 - ergostenol, ergostenol, ergostenol, α -ergostenol, β ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol,
 stigmasterol, stigmastanol, (3β) -7-dehydrocholesterol,

desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -sitosterol, β -sitosterol, γ -sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.

20. A composition of matter having a formula represented by

$$\begin{array}{c} {\rm H_{3}C-NH-\;(CH_{2}-CH_{2}-NH)_{\;x}-CH_{2}-CH_{2}-N-\;(CH_{2}-CH_{2}-NH)_{\;y}-CH_{2}-O-CO-Y}\\ \\ {\rm CO}\\ \\ {\rm I}\\ \\ {\rm Y} \end{array}$$

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wherein x is an integer of about 0 to about 1,200, y is an integer of about 0 to about 1,200, with the proviso that x + y is about 8 to about 1,200, and Y is a cholesterol residue.

21. A complex comprising a mixture of a nucleic acid and a composition of matter having a formula represented by

$$H_3C-NH-(CH_2-CH_2-NH)_x-CH_2-X-Y$$

wherein x is an integer of about 8 to about 1,200, X is a linker, and Y is a residue of a sterol comprising a 3-ol group.

- 22. The complex of claim 21 wherein x is about 581.
- 23. The complex of claim 21 wherein X is -O-CO-.
- 24. The complex of claim 21 wherein Y is a cholesterol

residue.

- 25. The complex of claim 21 wherein X is -O-CO- and Y is a cholesterol residue.
- 26. The complex of claim 21 wherein Y is a member selected from the group consisting of residues of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α-ergostenol, β-ergostenol, γ-ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmastanol, (3β)-7-dehydrocholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α₁-sitosterol, β-sitosterol, γ-sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.
 - 27. The complex of claim 21 wherein the nucleic acid comprises a plasmid.
 - 28. A complex comprising a mixture of a nucleic acid and a composition of matter having a formula represented by:

$$H_3C-NH-(CH_2-CH_2-NH)_x-CH_2-CH_2-N-(CH_2-CH_2-NH)_y-CH_2-OH$$
 | X | Y

wherein x is an integer of about 0 to about 1,200, y is an integer of about 0 to about 1,200, with the proviso that x + y is about 8 to about 1,200, X is a linker, and Y is a residue of a sterol comprising a 3-ol group.

- 5 29. The complex of claim 28 wherein x + y is about 581.
 - 30. The complex of claim 28 wherein X is -CO-.
 - 31. The complex of claim 28 wherein Y is a cholesterol residue.
- 32. The complex of claim 28 wherein X is -CO- and Y is a cholesterol residue.
 - 33. The complex of claim 28 wherein Y is a member selected from the group consisting of residues of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmastanol, (3 β)-7-dehydrocholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -sitosterol, β -sitosterol, γ -sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol,

and dehydroergosterol.

- 34. The complex of claim 28 wherein the nucleic acid comprises a plasmid.
- 34. A complex comprising a mixture of a nucleic acid and a composition of matter having a formula represented by

$$H_3C-NH-(CH_2-CH_2-NH)_x-CH_2-CH_2-N-(CH_2-CH_2-NH)_y-CH_2-X_1-Y_1$$
 | X_2 | Y_2

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wherein x is an integer of about 0 to about 1,200, y is an integer of about 0 to about 1,200, with the proviso that x+y is about 8 to about 1,200, X_1 and X_2 are linkers, and Y_1 and Y_2 are residues of a sterol comprising a 3-ol group.

- 15 35. The complex of claim 34 wherein x + y is about 581.
 - 36. The complex of claim 34 wherein X_1 is -O-CO- and X_2 is -CO-.
 - 37. The complex of claim 34 wherein Y_1 and Y_2 are cholesterol residues.

- 38. The complex of claim 34 wherein X_1 is -O-CO-, X_2 is -CO-, and Y_1 and Y_2 are cholesterol residues.
- 39. The complex of claim 34 wherein Y_1 and Y_2 are members independently selected from the group consisting of residues of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmastanol, (3β) -7-dehydrocholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -sitosterol, β -sitosterol, γ -sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.

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- 40. The complex of claim 34 wherein the nucleic acid comprises a plasmid.
- 15 41. A method of making an L-shaped linear polyethylenimine sterol conjugate comprising reacting a linear polyethylenimine having an average molecular weight of about 423 to about 50,000 and comprising a terminal hydroxyl group with a chloroformate ester of a sterol comprising a 3-ol group, thereby resulting in the L-shaped linear polyethylenimine sterol conjugate comprising the sterol covalently bonded to the terminal hydroxyl group.

42. The method of claim 41 wherein the sterol is a member selected from the group consisting of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmastanol, (3β) -7-dehydrocholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -sitosterol, β -sitosterol, γ -sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.

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- 43. The method of claim 41 wherein the sterol comprises cholesterol.
- 44. A method of making a T-shaped linear polyethylenimine sterol conjugate comprising:
- (a) reacting a linear polyethylenimine having an average molecular weight of about 423 to about 50,000 and comprising a terminal hydroxyl group and a plurality of secondary amine nitrogen atoms with a protecting reagent such that the protecting reagent bonds with the terminal hydroxyl group, resulting in a protected linear polyethylenimine;
 - (b) reacting a chloroformate ester of a sterol comprising a3-ol group with the protected linear polyethylenimine such that

the chloroformate ester of a sterol bonds with at least one of the plurality of secondary amine nitrogen atoms, resulting in a protected T-shaped linear polyethylenimine sterol conjugate; and

(c) deprotecting the protected T-shaped linear polyethylenimine sterol conjugate with a deprotecting reagent, resulting in the T-shaped linear polyethylenimine sterol conjugate.

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- 45. The method of claim 44 wherein the sterol is a member selected from the group consisting of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmastanol, (3β) -7-dehydrocholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -sitosterol, β -sitosterol, γ -sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.
 - 46. The method of claim 44 wherein the sterol comprises cholesterol.
- 47. The method of claim 44 wherein the protecting reagent comprises chlorotrimethylsilane and the protected linear

polyethylenimine comprises linear polyethylenimine trimethyl silane.

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- 48. The method of claim 47 wherein the deprotecting reagent comprises trifluoroacetic acid.
- 49. A method of making an LT-shaped linear polyethylenimine sterol conjugate comprising reacting a linear polyethylenimine having an average molecular weight of about 423 to about 50,000 and comprising a terminal hydroxyl group and a plurality of secondary amine nitrogen atoms with a chloroformate ester of a sterol comprising a 3-ol group such that chloroformate ester of a sterol bonds with the terminal hydroxyl group and at least one of the plurality of secondary amine nitrogen atoms, thereby resulting in the LT-shaped linear polyethylenimine sterol conjugate.
- 15 50. The method of claim 49 wherein the sterol is a member selected from the group consisting of cholesterol, cholestanol, coprosterol, epicholestanol, epicholesterol, ergostanol, α -ergostenol, β -ergostenol, γ -ergostenol, ergosterol, 22,23-dihydroergosterol, stigmasterol, stigmastanol, (3β) -7-dehydrocholesterol, desmosterol, allocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, campesterol, α_1 -

sitosterol, β -sitosterol, γ -sitosterol, lumisterol, pyrocalciferol, isopyrocalciferol, azacosterol, neoergosterol, and dehydroergosterol.

51. The method of claim 49 wherein the sterol comprises cholesterol.

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- 52. A method of delivering a nucleic acid into a mammalian cell comprising:
- (a) mixing the nucleic acid with an L-shaped, T-shaped, or LT-shaped linear polyethylenimine sterol conjugate to result in a complex;
- (b) contacting the mammalian cell with the complex such that the complex enters the mammalian cell, thereby delivering the nucleic acid into the mammalian cell.